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Keksinnön nimitys
Title of invention

"Gene cluster involved in nogalamycin biosynthesis, and its use in production of hybrid antibiotics"
(Nogalamysiinin biosynteesiin liittyvä geeniryhmittymä ja sen käyttö hybridiantibioottien tuotossa)

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Gene cluster involved in nogalamycin biosynthesis, and its use in production of hybrid antibiotics

Field of the invention

This invention relates to the gene cluster for nogalamycin biosynthesis derived from *Streptomyces nogalater*, and the use of the genes therein to obtain novel hybrid antibiotics for drug screening.

Background of the invention

Anthracyclines are antitumor antibiotics, mainly produced by *Streptomyces* sp. Daunomycin family of anthracyclines is commercially most important, since almost all of the around ten anthracyclines currently in clinical use, or in late clinical trials for cytotoxic drugs, belong to this family. Despite the long history of anthracyclines, three decades or so, the studies on their biosynthesis are still going on, and there is further interest to obtain novel molecules for the development of cancer chemo-therapeutics. A method currently used for finding novel molecules for drug screening is genetic engineering. Cloning the genes for anthracycline biosynthesis facilitates the production of hybrid anthracyclines, as well as their use in combinatorial biosynthesis to generate novel molecules.

Nogalamycin, which was first described by Bhuyan and Dietz in 1965, is an anthracycline antibiotic produced by *Streptomyces nogalater*. It is highly active against tumor cells, whereas toxic properties of this compound have prevented its progress to clinical trials (Bhuyan and Smith, 1975). However, menogaril (7-O-methylnogarol) is a semisynthetic derivative of nogalamycin, and its value in the treatment of cancer has been studied (e.g. Yoshida *et al.*, 1996), the interest being now mainly in Japan. Structurally nogalamycin (Fig. 1) differs from most other anthracyclines, as e.g. from the daunomycin family, in two noteworthy features: (i) The stereochemistry at position nine is opposite, and (ii) it has a sugar moiety, in which nogalamine is attached at position 1 by a typical glycosidic bond, but it is also attached to carbon 2 by an extraordinary C–C bond. Structural elucidation of

nogalamycin was reported by Wiley *et al.* (1977). Furthermore, biosynthetic studies of nogalamycin have been published by Wiley *et al.* in 1978 giving information of the building blocks: The aglycone moiety is built from ten acetates; the neutral sugar, nogalose, is derived from glucose; and methyl groups of both of the sugars, nogalamine and nogalose, are transferred from methionine. The origin of nogalamine was not clearly solved by Wiley, but most probably nogalamine is also derived from glucose.

Molecular cloning of biosynthesis genes for anthracyclines has facilitated the studies on molecular genetics, providing tools for rational modifications of the structures, while also for surprising combinations with other antibiotics. Most of the interest has focused on daunomycin biosynthesis genes, as reported in several publications (Lomovskaya *et al.*, 1998; Rajgarhia and Strohl, 1997 and references therein). Some genes for aclacinomycin biosynthesis from *S. galilaeus* (Fujii and Ebizuka, 1997) and for rhodomycin biosynthesis from *S. purpurascens* (Niemi *et al.*, 1994) have been cloned as well. We have cloned the biosynthesis genes for nogalamycin, and successfully used the genes for producing hybrid anthracyclines. Most of the genes are involved in polyketide pathway, being responsible for the formation of a tricyclic intermediate, and they are reported in Ylihonko *et al.*, 1996a and b, and by Torkkell *et al.*, 1997. Despite the advances in molecular cloning, the biosynthetic pathway from glucose to sugars found in anthracyclines is still mainly hypothetical.

Regarding the genes for deoxyhexose pathway, Madduri *et al.* (1998) have reported that a gene derived from avermectin biosynthesis cluster caused the production of hybrid anthracyclines altering the sugar moiety when transferred into an *S. peucetius* mutant. The product obtained was epirubicin, a commercially important anthracycline. In this case a hydroxy group in the daunosamine moiety was in the opposite stereochemistry due to the action of an avermectin biosynthesis gene. *S. galilaeus* has been used as the host to prepare hybrid anthracyclines using the genes derived from rhodomycin pathway from *S. purpurascens* (Niemi *et al.*, 1994), and from nogalamycin biosynthesis cluster from *S. nogalater* (Ylihonko *et al.*, 1996a). The genes for nogalamycin pathway were used to generate the hybrid anthracycline production in *S.*

steffisburgensis producing typically steffimycin (Kunnari *et al.*, 1997). Previously, biosynthesis genes for actinorhodin have been expressed in *S. galilaeus* resulting in the formation of aloesaponarin (Strohl *et al.*, 1991). These hybrid compounds were modified in the aglycone moiety.

Summary of the invention

The present invention concerns a gene cluster of *Streptomyces nogalater*, most of the genes of which are derived from the deoxyhexose pathway for nogalamine and nogalose. Expressing a DNA fragment of the said region in *S. galilaeus*, which produces aclacinomycins, hybrid anthracyclines are obtained, in which the aglycone moiety is derived from *S. galilaeus*, whereas the sugar moiety is characteristic neither to *S. nogalater* nor to *S. galilaeus*. Furthermore, when inserting the gene included in said cluster, encoding a cyclase for nogalamycin, into a suitable plasmid construction, nogalamycinone is obtained, which is the aglycone of nogalamycin. Since the stereochemistry of nogalamycin differs from most other anthracyclines, using this gene enables the preparation of C–9 stereoisomers of the anthracycline molecules.

Detailed description of the invention

The experimental procedures of the present invention are methods conventional in the art. The techniques not described in detail here are given in the manuals by Hopwood *et al.* "Genetic manipulation of Streptomyces: a laboratory manual" The John Innes Foundation, Norwich (1985) and by Sambrook *et al.* (1989) "Molecular cloning: a laboratory manual". The publications, patents and patent applications cited herein are given in the reference list in their entirety.

The present invention concerns particularly the gene cluster for nogalamycin biosynthesis (*Sno*5–cluster) causing the production of hybrid antibiotics with modifications in the sugar moiety. The invention concerns in specific the use of the genes for nogalamine/nogalose biosynthesis to generate hybrid antibiotics modified in sugar moieties. The invention also concerns the use of a specific cyclase gene

included in the gene cluster of the invention, to generate the C-9 stereoisomers of typical anthracyclines.

The gene cluster according to the present invention is linked to the earlier reported clusters for nogalamycin biosynthesis. The starting point of the present invention was the gene cluster for nogalamycin chromophor (International Patent Application WO 96/10581). Subsequently, we have found some genes for the deoxyhexose pathway of nogalamycin biosynthesis (Torkkell *et al.*, 1997), and a part of the fragment comprising said genes was used to clone the genes for this invention.

The biosynthesis genes for nogalamycin can be isolated from *Streptomyces* sp., particularly from *S. nogalater*, which produces nogalamycin. Species which produce nogalamycin-like anthracyclines can also be used, e.g. *S. violaceochromogenes* producing arugomycin (Kawai *et al.*, 1987), or *S. avidinii* producing avidinorubicin (Aoki *et al.*, 1991).

Genomic DNA of a *Streptomyces* strain carrying the genes for nogalamycin biosynthesis is used in preparing a genomic library. Suitable gene fragments for cloning may be obtained by any frequently digesting restriction enzyme. Typically *Sau*3AI is used. The isolated fragments could be inserted by ligation in any *Escherichia coli* vector such as a plasmid, a phagemid, a phage, or a cosmid. A cosmid vector is preferred since it enables the cloning of large DNA fragments. A cosmid vector such as pFD666 (ATCC No. 77286) is suitable for this purpose, as it enables cloning of the fragments of about 40 kb. The *Bam*HI site of pFD666, giving sticky ends to the *Sau*3AI fragments may be used for cloning. Commercially available kits may be used to pack the DNA in phage particles. Various *E. coli* strains can be used for the infection by the DNA packed. An appropriate *E. coli* strain is, e.g. XL1Blue MRF', which is deficient in several restriction systems.

Using *E. coli* as a host strain for the genomic library, hybridization is an advantageous screening strategy. The probe for hybridization may be any known fragment derived from the nogalamycin gene cluster, but a short fragment of about 1

kb derived from one end of the biosynthetic region previously cloned is preferred. Colonies for the genomic library are transferred for filter hybridization to membranes, preferably to nylon membranes. Since the average size for a genomic DNA fragment is 40 kb, 2300 colonies gave 99,99% probability to find the expanded region for nogalamycin biosynthesis. Any method for hybridization may be used but, in particular, the DIG System (Boehringer Mannheim, GmbH, Germany) is useful. Since the probe is homologous to the hybridized DNA, it is preferable to carry out the stringent washes of hybridization at 70°C in a low salt concentration according to Boehringer Mannheim's manual "DIG System User's Guide for Filter Hybridization". At least 80% homology is suggested to be needed for a DNA fragment to bind a probe in the conditions used for washes.

Using this protocol, seven clones out of about 5000 gave positive signals, and were picked up for DNA isolation. Restriction mapping is an appropriate technique for characterizing the clones. The positive clones may be digested with convenient restriction enzymes to demonstrate the physical linkage map of the DNA fragments. The cosmid used for cloning was a shuttle cosmid replicating in both *E. coli* and *Streptomyces* sp. However, the transfer of the recombinant cosmids in *S. lividans* TK24, which is a typically used laboratory strain in cloning *Streptomyces*, resulted in deletions, and was omitted. Instead, we rather used in the expression studies the plasmid pIJ486, a high copy number *Streptomyces* plasmid. However, any plasmid being able to stably replicate in *Streptomyces* may be used for this purpose.

Two *Bgl*II fragments of one of the clones were separately inserted into pIJ486 vectors, and the two plasmids obtained were transferred into a primary host, *S. lividans* TK24. The recombinant plasmids obtained (pSY42 and pSY43), containing a 10 kb and a 7kb fragment from *S. nogalater* genomic DNA, respectively, were isolated from the primary host and further introduced into other *Streptomyces* strains by protoplast transformation. The recombinant plasmid containing the 10 kb fragment caused the production of hybrid anthracyclines in the *S. galilaeus* mutant strain H039, which endogenously produces aklavinone–rhodinose–rhodinose–rhodinose. A few other *S. galilaeus* strains (H075, H026, H063) mutated in deoxyhexose pathway

for sugars in aclacinomycin were used in transformation, and new hybrid compounds were obtained. Since the structure of nogalamycin is almost unique among anthracyclines, the plasmids could be transferred to other anthracycline–producing strains, such as *S. peucetius*, which produces daunomycin, and *S. purpurascens*, which produces rhodomycins, to modify the structures of the characteristic antibiotics.

As the cloned cluster was linked to nogalamycin biosynthesis region already known, its ability to generate the modification in sugar moiety suggested the presence of the genes for deoxyhexose pathway. However, sequencing is necessary to deduce the function of the genes in the cluster cloned. The DNA fragments of 10 kb and 7 kb were further inserted into the plasmid pSL1190 for subcloning. Sequencing strategies such as a deletion set of the DNA fragments, shotgun cloning or primer walking could be used, but we prefer to use restriction fragments for subcloning. Using ABI PRISM system (Perkin–Elmer) for sequencing it is possible to get 500 to 700 bases per one reaction, which means that about 1 kb fragments sharing overlapping bases are needed for sequencing. For this purpose, 27 subclones were constructed.

Sequencing of the flanked *Bgl*II fragments consisting of about 16000 bp revealed 15 complete ORFs. The sequence analysis can be made by any computer based program, such as GCG (Madison, Wisconsin, USA) package. According to the present invention the putative gene functions as deduced from the sequence homology of those available in the libraries are

aminotransferase (*sno*gI), not completed

1. dTDP–glucose synthase (*sno*gJ)
2. aminomethyl transferase (*sno*gA)
3. polyketide cyclase, (*sno*aM)
4. a gene of deoxyhexose pathway, unknown (*sno*gN)
5. hydroxylase, (*sno*aG)
6. dTDP–4–dehydrorhamnose reductase (*sno*gC)
7. dTDP–glucose 4,6–dehydratase (*sno*gK)
8. NAME cyclase (*sno*aL)

9. unknown (*sno*K)
10. glycosyl transferase, GTF (*sno*gD)
11. unknown (*sno*W)
12. glycosyl transferase, GTF (*sno*gE)
13. unknown (*sno*L)
14. unknown (*sno*O)
15. C-7 ketoreductase (*sno*aF)

unknown (*sno*N), not completed

Gene designations: g means that the gene involved in biosynthesis of the glycosidic proportion including glycosyl transferases, whereas a points out that the gene is needed for the formation of the aglycone moiety.

Considering the proposed biosynthetic pathway for nogalamycin shown in Fig 3. we are able to cause several changes for the structures of antibiotics by the genes identified, including *sno*aL, responsible for the cyclization of the fourth ring of the aglycone moiety while determining the stereochemistry of the anthracyclinone, and the genes affecting the formation of nogalamine and nogalose (*sno*gJ, *sno*gK, *sno*gN, *sno*gC, *sno*gA), and, in addition, the genes responsible for joining the sugar residues to the aglycone moiety (*sno*gD and *sno*gE).

These genes could be separately inserted in a vector using suitable restriction sites, or by amplifying the genes by PCR. The fragments may contain an intrinsic promoter, or a promoter may be separately cloned. It is advantageous to use a vector carrying a promoter to allow expression of the genes in a *Streptomyces* strain. The plasmid pIJE486 contains a promoter *erm*E for erythromycin resistance gene, allowing constitutive expression of the genes inserted in a correct orientation. Special attention is drawn to the gene encoding a cyclase for the aliphatic ring, but any gene of said cluster may be expressed in *Streptomyces* hosts. The said cyclase converts the stereochemistry at C9 of auramycinone in TK24, if inserted into the plasmid possessing the other genes for auramycinone biosynthesis, except the cyclase responsible for the typical stereochemistry of anthracyclines.

Streptomyces strains, in particular *S. galilaeus*, carrying the recombinant plasmids are cultivated in media wherein antibiotics are produced. The hybrid compounds are extracted with organic solvents from the culture broth, and the compounds are separated and purified using chromatographic techniques.

According to this invention *S. galilaeus* H039 carrying the plasmid pSY42 and designated as H039/pSY42 produces aklavinone-4'-epi-2-deoxyfucose in E1 medium supplemented with thiostrepton to give selection pressure for the plasmid containing strains.

S. lividans TK24 carrying the plasmid pSY15c containing the genes for the nogalamycin chromophor and the genes for a cyclase (*sno*aL) and a ketoreductase (*sno*aF), was cultivated in E1 medium supplemented with thiostrepton. The compound 9-epi-auramycinone was produced, and this structure is now called nogalamycinone. Any DNA fragment of the invention subcloned from a 17 kb nogalamycin biosynthesis region can be inserted in a vector replicating in *Streptomyces*, and the products may be produced by fermentation of the plasmid containing strains.

Brief description of the drawings

Fig. 1 shows the structures of nogalamycin, daunomycin and aclacinomycin.

Fig. 2 is a diagram of the gene cluster (*Sno*5) of the invention for nogalamycin biosynthesis.

Fig. 3 describes the proposed biosynthesis pathway for nogalamycin.

Fig. 4 shows a diagram of the plasmid pSY15c. The genes *sno*aL (**aL**) and *sno*aF (**aF**) shown black are inserted in the plasmid pSY15 to give pSY15c. **aL** represents a cyclase *sno*aL and **aF** is for C-7 ketoreductase *sno*aF. pSY15 (WO 96/10581) generates the production of a tricyclic intermediate for

nogalamycin biosynthesis in *S. lividans*. The abbreviations **a1**, **a2** and **a3** refer to the genes *sno*a1, *sno*a2 and *sno*a3, respectively, for minimal PKS. **rA** is the *snor*A gene for an activator, **aB** is the *sno*aB gene for oxygenase, **aC** is the *sno*aC gene for methylase, **aD** is the *sno*aD gene for polyketide ketoreductase and **aE** is the *sno*aE for aromatase. **gF** (the *sno*gF gene) and **gG** (the *sno*gG gene) involved in the deoxyhexose pathway are not functional in the construct. **aph** is an aminoglycoside phosphotransferase gene, and **tsr** is a thiostreptone resistance gene.

Examples to further illustrate the invention are given hereafter.

EXPERIMENTAL

Materials used

Restriction enzymes used were purchased from Promega (Madison, Wisconsin, USA) or Boehringer Mannheim (Germany), and alkaline phosphatase from Boehringer Mannheim, and used according to the manufacturers' instructions. Proteinase K was purchased from Promega and lysozyme from Sigma (St. Louis, USA). Hybond™-N nylon membranes used in hybridization were purchased from Amersham (Buckinghamshire, England), DIG DNA Labelling Kit and DIG Luminescent Detection Kit from Boehringer Mannheim. Qiaquick Gel Extraction Kit from Qiagen (Hilden, Germany) was used for isolating DNA from agarose.

Bacterial strains and their use

Escherichia coli XL1 Blue MRF' was used for cloning.

Streptomyces nogalater ATCC 27451; the gene cluster of nogalamycin biosynthesis was cloned from this strain.

The host strains to express the genes cloned were:

Streptomyces lividans TK24, also used as a primary host to clone DNA propagated in *E. coli*.

Streptomyces galilaeus H039, produces aklavinone–rhodinose–rhodinose–rhodinose

Streptomyces galilaeus H026, produces aclacinomycin N, ACMN, (aklavinone–rhodosamine–2–deoxyfucose–rhodinose)

Streptomyces galilaeus H063, produces aklavinone

Streptomyces galilaeus H075, produces aklavinone–rhodosamine–2–deoxyfucose–2–deoxyfucose

The detailed description of the mutants H039 and H026 is given in Ylihonko *et al.* (1994) and of H075 in the FI patent application No. 981062 (Ylihonko *et al.*, 1998). H063 has not been described in the literature but it was obtained by NTG mutagenesis of *S. galilaeus*, and selected to be used as the host strain in the hybrid compound production, as it accumulates aklavinone without any sugar residues.

Plasmids

E. coli – Streptomyces shuttle cosmid pFD666 (ATCC 77286) was used for cloning the chromosomal DNA. *E. coli* cloning vectors pSL1190 (Pharmacia) and pUC19 were used for preparing the subclones.

pIJ486 is a high copy plasmid vector provided by prof. Sir David Hopwood, John Innes Centre, UK (Ward *et al.*, 1986)

pIJE486 is a vector containing *erm*E gene in the polylinker of pIJ486 (Bibb *et al.*, 1985).

pSY15 is a pIJ486 based plasmid construct, wherein the genes of polyketide pathway for nogalamycin biosynthesis were cloned (Ylihonko *et al.*, 1996a).

Nutrient media and solutions

For cultivation of *S. nogalater* for total DNA isolation TSB medium was used. Lysozyme solution (0.3 M sucrose, 25 mM Tris, pH 8 and 25 mM EDTA pH 8) was used in isolation of total DNA. TE buffer (10 mM Tris, pH 8.0 and 1mM EDTA) was used to dissolve the DNA.

TRYPTONE–SOYA BROTH (TSB)

Per litre: Oxoid Tryptone Soya Broth powder 30 g.

ISP4

Bacto ISP–medium 4, Difco; 37 g/l.

E1 Per litre in tap water:

glucose	20 g
soluble starch	20 g
Farmamedia	5 g
yeast extract	2.5 g
$K_2HPO_4 \bullet 3H_2O$	1.3 g
$MgSO_4 \bullet 7H_20$	1 g
NaCl	3 g
$CaCO_3$	3 g

pH adjusted to 7.4 before autoclaving

General methods

NMR data was collected with a JEOL JNM–GX 400 spectrometer at the ambient temperature. ^1H and ^{13}C NMR samples were internally referenced to TMS.

The anthracycline metabolites were detected by HPLC (LaChrom, Merck Hitachi, pump L–7100, detector L–7400 and integrator D–7500) using a LiChroCART RP–18 column (4.6x250mm). Acetonitrile:potassium hydrogen phosphate buffer (60 mM, pH 3.0 adjusted with citric acid) was used as the mobile phase. Gradient system starting from 65% to 30% of potassium dihydrogen phosphate buffer was used to separate the compounds. The flow rate was 1 ml/min and the detection was effected at 430 nm.

ISP4 plates supplemented with thiostrepton (50 μg/ml) were used to maintain the plasmid carrying cultures.

Example 1. Cloning the gene cluster for nogalamycin biosynthesis

1.1 Cosmid library

For the isolation of total DNA, *Streptomyces nogalater* (ATCC 27451) was grown for three days in 50 ml of TSB medium supplemented with 0.5% of glycine. The cells were harvested by centrifuging for 15 min at 3900 x g in 12 ml Falcon tubes, and the cells were stored at -20°C. Cells from a 12 ml sample of the culture were used to isolate the DNA. 5 ml of lysozyme solution containing 5 mg of lysozyme/ml was added onto the cells, incubated for 20 min at 37°C. 500 µl of 10% SDS containing 0.7 mg of proteinase K was added onto the cells and incubated for 80 min at 62°C, another 500 µl of 10% SDS containing 0.7 mg of proteinase K was added, and incubation was continued for 60 min. The sample was chilled on ice and 600 µl of 3M NaAc, pH 5.8 were added, and the mixture was extracted with equilibrated phenol (Sigma). The phases were separated by centrifuging at 1400 x g for 10 min. The DNA was precipitated from the water phase with equal volume of isopropanol to spool with a glass rod, and washed by dipping to 70% ethanol, air dried and dissolved in 500 µl of TE-buffer.

The chromosomal DNA was partially digested with *Sau*3AI. The DNA fragments were separated by agarose gel electrophoresis, and the fragments of 30 to 50 kb were cut from the 0.3% low gelling temperature SeaPlaque® agarose. The DNA bands were isolated from the gel by heating to 65°C, extracting with equal volume of equilibrated phenol, and the phases were separated by centrifuging for 15 min at 2500 x g. The phenol phase was extracted with TE buffer, centrifuged and the water phases were pooled. The DNA was precipitated by adding 0.1 volumes of NaAc, pH 5.8 and 2 volumes of ethanol at -20°C for 30 min, centrifuged for 30 min at 15 000 rpm in Sorvall RC5C centrifuge using SS-34 rotor with adapters for 10 ml tubes. The pellet was air dried and dissolved in 20 µl of TE buffer. The isolated fragments were ligated to pFD666 cosmid vector digested with *Bam*HI and dephosphorylated. The DNA was packed to phage particles, and infected to *E. coli* using Gigapack® III XL Packing Extract Kit according to the manufacturer's instructions.

1.2 Identification of the clones by hybridization

The infected cells were grown on LB plates containing 50 μg/ml kanamycin and transferred to Hybond™–N nylon membranes (Amersham). The membranes were handled according to the protocol described in Boehringer Mannheim's manual "The DIG System User's Guide for Filter Hybridization". The probe used to screen the colonies for an expanded nogalamycin gene cluster was a 1.07 kb *Sac*I fragment ~~from the cluster described earlier (Torkkell *et al.*, 1997). The plasmid carrying the~~ probe was digested with *Sac*I, and the fragment was separated from the vector by ~~agarose gel electrophoresis and isolated from the gel using Qiaquick Gel Extraction~~ Kit (Qiagen). The probe was labelled by digoxygenin using random prime labelling system according to Boehringer Mannheim's manual "The DIG System User's Guide for Filter Hybridization". 5000 colonies were screened by hybridization at 70°C using the probe described. Positive colonies were detected using DIG Luminescent Detection Kit (Boehringer Mannheim). Seven colonies gave a positive signal. Cosmids from the positive clones were isolated from a 5ml culture by alkaline lysis method. Restriction analysis showed that the cloned fragments overlapped each other representing at least 60 kb of the continuous DNA. The positive clones obtained were ~~designated as pFD*Sno*1 to pFD*Sno*7.~~

1.3. Subcloning the fragments for sequencing

Clone No. 5, designated as pFD*Sno*5, was digested with *Bgl*II, and for subcloning two fragments of about 10 kb and 7 kb were isolated and ligated to pSL1190 digested with *Bgl*II and dephosphorylated. The plasmids obtained were named as pSn42 and pSn43, respectively. These two fragments cover the DNA region flanked to the previously characterized area of nogalamycin biosynthesis cluster. To determine the nucleotide sequence of the whole 17 kb region cloned in pSn42 and pSn43 the convenient restriction sites were used to subclone the fragments to the vector pUC19 or pSL1190 giving 16 subclones from the insert of pSn42 and 11 subclones of pSn43.

E. coli XL1 Blue MRF' cells were cultivated overnight at 37 °C in 5 ml of LB–medium supplemented with 50 μg/ml of ampicillin. To isolate plasmids for

sequencing reactions Wizard Plus Minipreps DNA Purification System kit of Promega, or Biometra silica spin plasmid miniprep kit of Biomedizinische Analytik Gmbh were used according to the manufacturers' instructions.

DNA sequencing was performed using the automatic ABI DNA sequenator (Perkin-Elmer) according to the manufacturer's instructions.

1.4 Sequence analysis and the deduced functions of the genes

Sequence analyses were effected using the GCG sequence analysis software package (Version 8; Genetics Computer Group, Madison, Wisconsin, USA). The translation table was modified to accept also GTG as a start codon. Codon usage was analysed using published data (Wright and Bibb 1992).

According to the CODONPREFERENCE program the sequenced DNA fragment contained 15 complete open reading frames (ORFs), and the 5' end of other two ORFs in the both ends of the fragment according to the invention. The functions of the genes were concluded by comparing the amino acid sequences translated from their base sequences to the known protein sequences in the data banks. The results are shown in Table 1. The positions given refer to the appended sequence listing. The amino acid sequences of the peptides are given in SEQ ID NO:2 to SEQ ID NO:18.

Table 1

Gene	Position	Amino acids (SEQ ID NO)	Deduced function	Remarks
*snog*I	–1027 compl	>342 (2)	aminotransferase	5' end
*snog*J	1192–2073	293 (3)	dTDP–glucose synthase	
*snog*A	2106–2822 compl	238 (4)	aminomethyl transferase	
*snoa*M	2826–3800 compl	324 (5)	a polyketide cyclase	
*snog*N	3799–5025	408 (6)	*dnr*Q homology (Otten *et al.*, 1995), unknown	
*snoa*G	5088–6356	422 (7)	hydroxylase	
*snog*C	6334–7209 compl	291 (8)	dTDP–4–dehydrorhamnose reductase	
*snog*K	7245–8297 compl	350 (9)	dTDP–glucose–4,6–dehydratase	
*snoa*L	8537–8941	134 (10)	NAME cyclase (nogalonic acid methyl ester)	
snoK	8992–9699	235 (11)	unknown	
*snog*D	9745–10917 compl	390 (12)	glycosyl transferase	
snoW	11057–11884	275 (13)	unknown	
*snog*E	11928–*	>424 (14)	glycosyl transferase	
snoL	13335–13754 compl	139 (15)	unknown	
snoO	13974–14441	155 (16)	homologous to *mtm*X of mithramycin cluster	
*snoa*F	14532–15377	281 (17)	C–7 ketoreductase analogous to aklaviketone ketoreductase	
snoN	15450–*	>190 (18)	unknown	5' end

*, nucleotide sequence of about 100 bp, not known

1.5 Expression cloning

The 10 kb *Bgl*II fragment from pFD*Sno5* was cloned into the plasmid pIJ486 and the plasmid obtained was named as pSY42. Correspondingly, the 7 kb *Bgl*II fragment from pFD*Sno5* was cloned into the plasmid pIJE486, and the plasmid pSY43 was obtained. Plasmid pSY42 was introduced into *S. lividans* strain TK24 by protoplast transformation, isolated from it and further introduced into *S. galilaeus* mutant H039, and after propagation in H039, transferred to other *S. galilaeus* mutants blocked in the deoxyhexose pathway for characteristic sugars of aclacinomycins (H075, H026, and H063). E1 medium was used for anthracycline production, and the products were extracted from the culture with toluene:methanol (1:1) at pH 7. Anthracycline metabolites were analyzed by HPLC. The products of the mutants H039, H026, H063 and H075 carrying pSY42 differed from those obtained by the mutants without the plasmid.

According to the sequence analysis pSY42 contained a cyclase designated as NAMEC (nogalonic acid methyl ester cyclase), and in pSY43 a ketoreductase gene was identified. Expression constructions were prepared which contained all the genes needed for the formation of nogalamycin aglycone. A 1.4 kb *Bam*HI–*Sac*I fragment from pSY42 (containing NAMEC) and a 1.1 kb *Mlu*I–*Kpn*I fragment from pSY43 carrying the gene for a ketoreductase of C–7 keto group were ligated to pSY15 linearized by *Sac*I, to form the plasmid pSY15c (Fig. 4). Plasmid pSY15c was introduced into *S. lividans* TK24, and the strain TK24/pSY15c was cultivated in E1 medium supplemented with thiostrepton. An aglycone compound was produced, and this structure is now called nogalamycinone.

Example 2. Compounds generated by the *sno5*–cluster

2.1 Production and purification of the products derived from H039/pSY42 and TK24/pSY15c

The seed culture, 180 ml of E1 culture of the plasmid containing strain, H039/pSY42 or TK24/pSY15c, was obtained by cultivating the strain in three 250 ml Erlenmeyer flasks containing 60 ml of E1 medium supplemented with thiostrepton (5 μg/ml) for

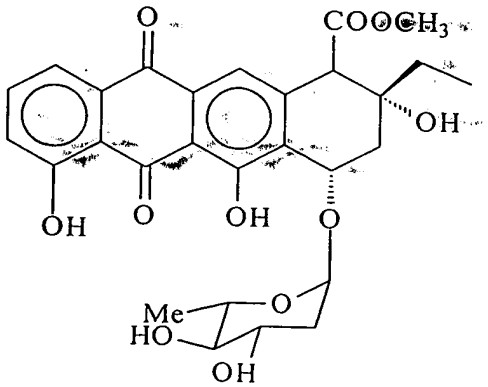
four days at 30°C, 330 rpm. The combined culture broths (180 ml) were used to inoculate 13 l of E1 medium in a fermentor (Biostat E). Fermentation was carried out for seven days at 28°C (330 rpm, aeration: 450 l/min).

The cells were harvested by centrifuging. 2.6 l of methanol was used to break the bacterial cells and to extract anthracycline metabolites accumulated. The anthracycline metabolites were extracted using 2 l of dichloromethane at pH 6. The organic layer was evaporated to dryness. The viscous residue was flashed through a polyamide (11) column using water:methanol from 1:9 to 0:10 as the eluent. Pooled fractions containing the compounds were further purified on a Merck–Hitachi HPLC using preparative reversed phase column (LichroCART RP–18, 5 μm) with mobile phase acetonitrile:1 % AcOH in water (1:1). Evaporation of acetonitrile gave pure products as yellow powders dried under vacuum.

2.2 Structural elucidation of the compounds derived from H039/pSY42 and from TK24/pSY15c

NMR analysis included NON, BMC, NOE, DEPT and HMBC techniques. Protons were assignated using NOESY and 2D pTOCSY techniques and carbons using DEPT and HMBC techniques.

As deduced from the data given in Tables 2 and 3, the structures revealed were aklavinone–4'–epi–2–deoxyfucose from the culture of H039/pSY42, and 9–epi–auramycinone (=nogalamycinone) from the culture of TK24/pSY15c. The chemical structures of the compounds are shown below in Formula I and Formula II, respectively.



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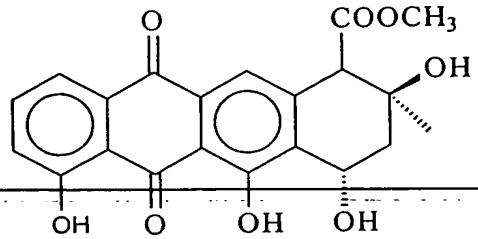

(II)

Deposited microorganisms

The following microorganisms were deposited according to the Budapest Treaty at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig, Germany.

Microorganism	Accession number	Date of deposit
S. lividans TK24/pSY42 carrying the plasmid pSY42	DSM 12451	14 October 1998
S. lividans TK24/pSY43 carrying the plasmid pSY43	DSM 12452	14 October 1998

Table 2. **^{1}H and ^{13}C assignations of the compound aklavinone–4'–epi–2–deoxyfucose (Formula I).**

Site	^{1}H	^{13}C
1	7.74, 1H, dd, 7.5, 1.3	120.1
2	7.68, 1H, dd, 8.4, 7.5	137.3
3	7.27, 1H, dd, 8.3, 1.3	124.6
4	–	161.9
4–OH	11.70, 1H, s	–
4a	–	115.4
5	–	192.3
5a	–	114.4
6	–	162.4
6–OH	12.46, 1H, s	–
6a	–	130.9
7	5.18, 1H, dd, 4.3, 3.1	71.3
8A	2.51, 1H, dd, 15.0, 4.3	33.9
8B	2.32, 1H, dd, 15.0, 3.1	–
9	–	72.1
9–OH	4.58, 1H, s	–
10	4.02, 1H, s	56.9
10a	–	142.4
11	7.40, 1H, s	120.8
11a	–	133.1
12	–	180.7
12a	–	132.6
13A	1.73, 1H, dq, 14.2, 7.4	32.0
13B	1.51, 1H, dq, 14.2, 7.4	–
14	1.10, 3H, t, 7.4	6.7
15	–	171.1
16	3.69, 3H, s	52.5
1′	5.41, 1H, d, 3.5	101.7
2′a	1.75, 1H, ddd, 12.8, 11.2, 3.4	37.7
2′e	2.19, 1H, dd, 12.8, 5.3	–
3′	3.71, 1H, ddd, 12.0, 9.0, 5.3	69.0
4′	3.14, 1H, dd, 9.1, 9.0	78.1
5′	3.88, 1H, dq, 9.1, 6.2	68.8
6′	1.36, 3H, d, 6.2	17.6

Table 3. **^{1}H and ^{13}C assignations of 9–epi–auramycinone (Formula II).**

Site	^{1}H	^{13}C
1	7.76, 1H, dd, 7.5, 1.2	119.8
2	7.67, 1H, dd, 8.3, 7, 5	137.4
3	7.28, 1H, dd, 8.3, 1.2	124.8
4	–	162.5
4–OH	11.86, 1H, s	–
4a	–	115.6
5	–	192.7
5a	–	114.6
6	–	160.9
6–OH	12.76, 1H, s	–
6a	–	134.1
7	5.40, 1H, t, 7.0	64.0
8A	2.66, 1H, dd, 13.9, 7.0	40.9
8B	1.89, 1H, dd, 13.9, 7.1	–
9	–	70.5
9–OH	3.49, 1H, brs	–
10	3.93, 1H, d, 0.8	56.0
10a	–	142.1
11	7.51, 1H, d, 0.8	120.1
11a	–	133.3
12	–	180.9
12a	–	132.1
13	1.44, 3H, s	28.7
14	–	173.0
15	3.90, 3H, s	52.6

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(B) STREET: Kairiskulmantie 10
(C) CITY: Piispanristi
(E) COUNTRY: Finland
(F) POSTAL CODE (ZIP): FIN-20760

(ii) TITLE OF INVENTION: Gene cluster involved in nogalamycin biosynthesis, and its use in production of hybrid antibiotics

(iii) NUMBER OF SEQUENCES: 18

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16020 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: *Streptomyces nogalater* ATCC 27451

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 342 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogI*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Thr Val His Val Trp Asp Tyr Leu Pro Glu Tyr Glu Leu Glu Arg
1 5 10 15

Glu Asp Ile His Asp Ala Val Glu Thr Val Phe Arg Ser Gly Arg Leu
20 25 30

Val Leu Gly Glu Ser Val Arg Gly Phe Glu Ser Glu Phe Ala Ser Phe
35 40 45

Gln Gly Val Gly His Ala Val Gly Val Asp Asn Gly Thr Asn Ala Val
50 55 60

Lys Leu Gly Leu Gln Ala Leu Gly Val Gly Pro Gly Asp Glu Val Val
65 70 75 80

Thr Val Ser Asn Thr Ala Ala Pro Thr Val Val Ala Ile Asp Ser Ala
85 90 95

Gly Ala Thr Pro Val Phe Val Asp Val Arg Glu Glu Asp Tyr Leu Met
100 105 110

Asp Thr Ser Gln Val Glu Ala Val Leu Thr Pro Arg Thr Arg Cys Leu
115 120 125

Leu Pro Val His Leu Tyr Gly Gln Cys Val Asp Met Ala Pro Leu Arg
130 135 140

Asp Leu Ala Ala Arg His Asn Leu Val Ile Leu Glu Asp Cys Ala Gln
145 150 155 160

Ala His Gly Ala Arg Arg His Gly Arg Leu Ala Gly Ser Thr Gly Asp
165 170 175

Ala Ala Ala Phe Ser Phe Tyr Pro Thr Lys Val Leu Gly Ala Tyr Gly
180 185 190

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Asp Gly Gly Ala Val Leu Thr Asp Asp Glu Arg Val Ala Asp Arg Leu
        195                 200                 205

Arg Arg Leu Arg Tyr Tyr Gly Met Glu Ser Arg Tyr Tyr Val Val Glu
    210                 215                 220

Thr Pro Gly His Asn Ser Arg Leu Asp Glu Val Gln Ala Glu Ile Leu
225                 230                 235                 240

Arg Arg Lys Leu Ser Arg Leu Pro Ser Tyr Ile Glu Ala Arg Arg Ala
                245                 250                 255

Val Ala Arg Arg Tyr Glu Glu Gly Leu Ala Asp Thr Gly Leu Leu Leu
            260                 265                 270

Pro Arg Thr Ala Gln Gly Asn Glu His Val Tyr Tyr Val Tyr Val Val
        275                 280                 285

Arg His Pro Arg Arg Asp Ala Val Leu Glu Ala Leu Arg Ala Ser Tyr
    290                 295                 300

Asp Ile Ala Leu Asn Ile Ser Tyr Pro Trp Pro Val His Thr Met Thr
305                 310                 315                 320

Gly Phe Ser His Leu Gly Tyr Ala Lys Gly Ser Leu Pro Val Thr Glu
                325                 330                 335

Ala Leu Ala Asp Glu Ile
            340
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 298 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogJ*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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Thr Thr Leu Ala Val Ser Lys Gln Leu Leu Pro Val Gly Asp Lys Pro
            20                  25                  30

Met Ile Tyr Tyr Pro Leu Ser Val Leu Met Leu Ala Gly Val Thr Asp
        35                  40                  45

Ile Leu Ile Ile Ser Thr Pro His Glu Leu Pro Arg Met Arg Arg Leu
    50                  55                  60

Phe Gly Asp Gly Ala Gln Leu Gly Leu Arg Leu Ala Tyr Ala Glu Gln
65                  70                  75                  80

Glu Lys Pro Arg Gly Ile Ala Glu Ala Phe Leu Ile Gly Ala Asp His
                85                  90                  95

Val Gly Ser Asp Ala Val Ala Leu Ala Leu Gly Asp Asn Ile Phe His
            100                 105                 110

Gly Ser Ser Phe Gln Gly Val Leu Arg Lys Glu Ala Glu Glu Leu Asp
        115                 120                 125
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Val Gly Glu Ala Asn Ala Ser Gly Arg Leu Val Ser Ile Glu Glu Lys
145                 150                 155                 160

Pro Val Arg Pro Arg Ser Asn Arg Ala Ile Thr Gly Leu Tyr Phe Tyr
                165                 170                 175

Asp Asn Glu Val Val Asp Ile Ala Arg Arg Leu Arg Pro Ser Ala Arg
            180                 185                 190

Gly Glu Leu Glu Ile Thr Asp Ile Asn Arg Thr Tyr Met Glu Arg Gly
        195                 200                 205

Arg Ala Arg Leu Val Asp Leu Gly Arg Gly Phe Ala Trp Leu Asp Thr
    210                 215                 220

Gly Thr Pro Glu Ser Leu Leu Gln Ala Ser Gln Tyr Val Ser Ala Leu
225                 230                 235                 240

Glu Glu Arg Gln Gly Ile Arg Ile Ala Cys Ile Glu Glu Val Ala Leu
                245                 250                 255

Arg Met Gly Phe Ile Asn Ala Gln Ala Cys Tyr Glu Leu Gly Ala Arg
            260                 265                 270

Leu Ser Gly Ser Gly Tyr Gly Gln Tyr Val Met Ala Ile Ala Glu Glu
        275                 280                 285

Cys Thr Gly Arg Val
    290
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 238 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogA*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Val Tyr Gly Arg Glu Leu Ala Asp Val Tyr Glu Met Val Tyr Arg Ser
1               5                   10                  15

Arg Gly Lys Ser Trp Ala Asp Glu Ala Glu Arg Val Thr Ala Glu Ile
            20                  25                  30

Arg Ser Arg Arg Pro Gly Ala Arg Ser Leu Leu Asp Val Ala Cys Gly
        35                  40                  45

Thr Gly Ala His Leu Glu Ala Phe Arg Gly Leu Phe Ala His Thr Glu
    50                  55                  60

Gly Leu Glu Leu Ser Asp Glu Met Arg Ala Leu Ala Glu Arg Arg Leu
65                  70                  75                  80

Pro Gly Val Pro Val Arg Pro Gly Asp Met Arg Asp Phe Ala Leu Ser
                85                  90                  95

Gly Arg Phe Asp Ala Val Val Cys Leu Phe Cys Ser Ile Gly Tyr Leu
            100                 105                 110
```

```
Glu Thr Val Ala Asp Met Arg Ala Ala Val Arg Thr Met Ala Ala His
        115                 120                 125

Leu Val Pro Gly Gly Val Leu Val Val Glu Pro Trp Trp Phe Pro Glu
    130                 135                 140

Arg Phe Leu Glu Gly Tyr Val Ala Gly Asp Leu Ala Arg Gly Glu Gly
145                 150                 155                 160

Arg Thr Val Ala Arg Val Ser His Ser Thr Arg Gln Gly Arg Arg Thr
                165                 170                 175

Arg Met Glu Val Arg Phe Leu Val Gly Glu Ala Thr Gly Ile Arg Glu
            180                 185                 190

Phe Thr Glu Ile Asp Leu Leu Thr Leu Phe Thr Arg Glu Glu Tyr Leu
        195                 200                 205

Ala Ala Phe Glu Asp Ala Gly Cys Pro Ala Glu Phe Leu Asp Asp Gly
    210                 215                 220

Leu Thr Gly Arg Gly Leu Phe Val Gly Val Arg Gly Ala Gly
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 324 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoaM*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Thr Ala Ala Trp Gly Ala Pro Leu Tyr Pro Pro Trp Ile Pro Ala
1               5                   10                  15

Arg Pro Gly Arg Arg Arg Cys Gly Ala Gly Arg Arg Val Arg Cys Pro
            20                  25                  30

Pro Val Glu Pro Ala Ser Arg Pro Arg Gln Glu Gly Arg Val Ser Val
        35                  40                  45

Val Pro Ala Leu Arg Gln Pro Ser Pro Ser Thr Asn Pro Glu Val Arg
    50                  55                  60

Val Arg Leu Ile Asp Leu Ser Ser Pro Val Asp Ser Ser Gln Tyr Glu
65                  70                  75                  80

Pro Asp Pro Val Val His Asp Val Leu Thr Pro Arg Gln Gly Ala Glu
                85                  90                  95

His Met Cys Ala Glu Met Arg Glu His Phe Gly Val Glu Phe Ser Pro
            100                 105                 110

Asp Glu Leu Pro Asp Gly Glu Phe Leu Ser Leu Asp Arg Ile Thr Leu
        115                 120                 125

Thr Thr His Thr Gly Thr His Val Asp Ala Pro Ser His Tyr Gly Ser
    130                 135                 140

Arg Ala Leu Tyr Gly Asp Gly Val Pro Arg His Ile Asp Gln Met Pro
145                 150                 155                 160
```

```
Leu Glu Trp Phe Phe Gly Arg Gly Val Val Leu Asp Leu Thr Asp Ala
                165                 170                 175

Pro Thr Gly Thr Val Ser Ala Ala Arg Leu Glu Lys Glu Leu Ala Arg
            180                 185                 190

Thr Gly Cys Ala Leu Arg Pro Gly Asp Ile Val Leu Leu His Thr Gly
        195                 200                 205

Ala Gln Arg His Ala Gly Thr Pro Arg Tyr Phe Thr Asp Phe Ala Gly
    210                 215                 220

Leu Asp Gly Pro Ala Val Arg Met Leu Leu Asp His Gly Val Arg Val
225                 230                 235                 240

Ile Gly Thr Asp Ala Phe Ser Leu Asp Ala Pro Phe Gly His Ile Ile
                245                 250                 255

Asp Arg Tyr Arg Ala Thr Gly Asp Arg Ser Val Leu Trp Pro Ala His
            260                 265                 270

Val Val Gly Arg Glu Arg Glu Tyr Cys Gln Ile Glu Arg Leu Ala Asn
        275                 280                 285

Leu Asp Arg Leu Pro Val Ser Phe Gly Phe Arg Val Cys Cys Phe Pro
    290                 295                 300

Val Lys Val Ala Gly Ala Gly Ala Gly Trp Thr Arg Ala Val Ala Leu
305                 310                 315                 320

Val Asp Glu Asp
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogN*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Val Met Lys Leu Thr Asp Ser Glu Leu Gly Arg Ala Leu Leu Ser
1               5                   10                  15

Leu Arg Gly Tyr Gln Trp Leu Arg Gly Ile His His Asp Pro Tyr Ala
            20                  25                  30

Leu Leu Leu Arg Ala Glu Ser Asp Asp Pro Ala Gln Leu Gly Arg Leu
        35                  40                  45

Leu Arg Glu Arg Gly Arg Leu His Arg Ser Asp Thr Gly Thr Trp Val
    50                  55                  60

Thr Ala Asp His Ala Thr Ala Ser Arg Leu Leu Ala Asp Pro Arg Phe
65                  70                  75                  80

Val Leu Arg Arg Pro Pro Ala Gly Pro Ala Thr Gly Thr Gly Asp Val
                85                  90                  95

Met Pro Trp Glu Glu Ala Thr Leu Ser Asp Leu Leu Pro Leu Asp Glu
            100                 105                 110
```

```
Ala Arg Leu Thr Thr Asp Arg Ala Arg Cys Arg Arg Leu Gly Ala Thr
        115                 120                 125

Ala Ala Arg Ile Ala Ala Asp Gly Pro Val Ala Thr Arg Leu Ala Asp
    130                 135                 140

Leu Ala Gly Ala Arg Ala Glu Gln Val Arg Ser Thr Gly His Phe Asp
145                 150                 155                 160

Leu Arg Ala Asp Tyr Ala Leu Pro Tyr Ala Val Glu Pro Ala Cys Ala
                165                 170                 175

Leu Leu Gly Leu Pro Ala Gly Gln Cys Ser Leu Phe Gly Ala Phe Ser
            180                 185                 190

Pro Ala Val Leu Leu Asp Ala Thr Val Val Pro Pro Arg Leu Pro Glu
        195                 200                 205

Ala Arg Ala Leu Ile Ala Ser Thr Ala Glu Leu Thr Ala Leu Trp Pro
    210                 215                 220

Arg Leu Ala Pro Ser Leu Ser Lys Thr Val Pro Glu Asp Glu Ala Pro
225                 230                 235                 240

Asp Leu Phe Leu Leu Thr Ala Val Leu Leu Val Pro Ala Val Val His
                245                 250                 255

Leu Val Cys Glu Ala Val Ala Ala Leu Ser His Asp Pro Gly Gln Ala
            260                 265                 270

Gly Leu Leu Arg Asp Asp Pro Val Leu Ala Ala Pro Ala Val Glu Glu
        275                 280                 285

Thr Leu Arg His Ala Pro Pro Ala Arg Leu Phe Thr Leu His Ala Thr
    290                 295                 300

Gly Pro Glu Arg Val Ala Asp Val Asp Leu Pro Ala Gly Ala Glu Val
305                 310                 315                 320

Ala Val Val Val Ala Ala Ala His Arg Asp Pro Ser Trp Cys Pro Asp
                325                 330                 335

Pro Asp Arg Phe Asp Leu Thr Arg Asn Glu Arg His Leu Ala Leu Pro
            340                 345                 350

Pro Asp Leu Pro Leu Gly Ala Leu Ala Pro Leu Leu Arg Val Cys Ala
        355                 360                 365

Thr Ala Ala Val Ala Ala Leu Ala Ala Gly Leu Leu Pro Leu Arg Ala
    370                 375                 380

Val Gly Pro Pro Val Arg Arg Leu Arg Ala Pro Val Thr Arg Ser Val
385                 390                 395                 400

Leu Arg Phe Pro Val Ala Pro Cys
                405
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 422 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoaG*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Asp Asn Arg Glu Thr Val Arg Pro Val Ser Val Cys Arg Val Cys
1               5                  10                  15

Gly Gly Asn Asp Trp Gln Asp Val Val Asp Phe Gly Asp Val Pro Leu
            20                  25                  30

Ala Asn Gly Phe Leu Ser Pro Ala Asp Ser Tyr Glu Asn Glu Arg Arg
        35                  40                  45

Tyr Pro Leu Gly Val Leu Ser Cys Arg Ala Cys Arg Leu Met Ser Leu
    50                  55                  60

Thr His Val Val Asp Pro Glu Val Leu Tyr Arg Asp Tyr Ala Tyr Thr
65                  70                  75                  80

Thr Pro Asp Ser Glu Met Ile Thr Gln His Met Arg His Ile Thr Ala
                85                  90                  95

Leu Cys Arg Thr Arg Phe Glu Leu Pro Pro Asp Ser Leu Val Val Glu
            100                 105                 110

Leu Gly Ser Asn Thr Gly Arg Gln Leu Met Ala Phe Arg Glu Ala Gly
        115                 120                 125

Met Arg Thr Leu Gly Val Asp Pro Ala Arg Asn Leu Thr Asp Val Ala
    130                 135                 140

Arg Arg Asn Gly Ile Glu Thr Phe Pro Asp Phe Phe Ser His Asp Val
145                 150                 155                 160

Ala Arg Thr Ile Arg Arg Asp His Gly Gln Ala Arg Leu Val Leu Gly
                165                 170                 175

Arg His Val Phe Ala His Ile Asp Asp Val Ser Asp Ile Ala Ala Gly
            180                 185                 190

Val Arg Glu Leu Leu Ser Pro Asp Gly Val Phe Ala Ile Glu Val Pro
        195                 200                 205

Tyr Val Leu Asp Leu Leu Glu Lys Val Ala Phe Asp Thr Ile Tyr His
    210                 215                 220

Glu His Leu Ser Tyr Phe Thr Met Arg Ser Phe Val Thr Leu Phe Ala
225                 230                 235                 240

Arg His Gly Leu Arg Val Leu Asp Val Glu Arg Phe Gly Val His Gly
                245                 250                 255

Gly Ser Val Leu Val Phe Val Gly His Glu Asp Gly Pro Trp Pro Glu
            260                 265                 270

Arg Pro Ser Val Pro Glu Leu Leu Arg Val Glu Arg Gln Arg Gly Leu
        275                 280                 285

Tyr Asp Asp Ala Thr Tyr Arg Thr Phe Ala Gln Arg Ile Glu Arg Val
    290                 295                 300

Arg Thr Glu Leu Pro Glu Leu Leu Arg Ser Leu Val Ala Gln Gly Lys
305                 310                 315                 320

Arg Ile Val Gly Tyr Gly Ala Pro Ala Lys Gly Asn Thr Ile Leu Thr
                325                 330                 335

Val Cys Gly Leu Gly Leu Lys Glu Leu Glu Tyr Cys Thr Asp Thr Thr
            340                 345                 350
```

```
Glu Leu Lys Gln Gly Arg Val Leu Pro Gly Thr His Ile Pro Val His
        355                 360                 365

Ala Pro Glu His Ala Lys Glu His Ile Pro Asp Tyr Tyr Leu Leu Leu
    370                 375                 380

Ala Trp Asn Tyr Ala Thr Glu Ile Leu Asp Lys Glu Thr Ala Phe Arg
385                 390                 395                 400

Asp Asn Gly Gly Arg Phe Ile Val Pro Ile Pro Arg Pro Ser Ile Leu
                405                 410                 415

Thr Ser Pro Ser Gly Ser
            420
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 291 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogC*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Leu Ala Arg His Leu Thr Ala Ala Leu Ala Glu Thr Gly Arg Ser
1               5                  10                  15

Arg Pro Ala Ala Glu Ala Val Val Leu Gly Arg Arg Ala Leu Asp Ile
            20                  25                  30

Thr Asp Gly Arg Ala Val Asp Ala Ala Phe Ala Ala His Arg Pro Arg
        35                  40                  45

Val Val Val Asn Cys Ala Ala Phe Thr Asp Val Asp Gly Ala Glu Ser
    50                  55                  60

Arg Trp Ala Glu Ala Met Arg Val Asn Gly Gly Gly Pro Arg Leu Leu
65                  70                  75                  80

Ala Arg Arg Cys Ala Arg His Gly Val Arg Leu Ile His Val Ser Thr
                85                  90                  95

Asp Tyr Val Phe Pro Gly Asp Thr Arg Ser Pro Tyr Gly Glu Ser Asp
            100                 105                 110

Ala Pro Gly Pro Arg Thr Val Tyr Gly Arg Ser Lys Leu Ala Gly Glu
        115                 120                 125

Arg Ala Val Leu Ser Leu Leu Pro Asp Thr Gly Thr Val Val Arg Thr
    130                 135                 140

Ala Trp Leu Tyr Gly Gly Gln Gly Arg Ser Phe Val Arg Thr Met Leu
145                 150                 155                 160

Glu Arg Ala Pro Asp Asp Gly His Val Asp Val Val Asn Asp Gln Trp
                165                 170                 175

Gly Gln Pro Thr Trp Ala Gly Asp Val Ala Arg Leu Leu Val Thr Leu
            180                 185                 190

Ala Arg Thr Pro Pro Asp Arg Ala Arg Gly Ile Phe His Ala Thr Asn
        195                 200                 205
```

```
Ala Gly Ala Ala Thr Trp Tyr Glu Leu Ala Arg Glu Val Phe Arg Leu
    210                 215                 220

Ala Gly Ala Asp Pro Glu Arg Val Arg Pro Val Ala Thr Ala Asp Arg
225                 230                 235                 240

Pro Gly Pro Ala Pro Arg Pro Ala Cys Thr Val Leu Gly His Asp Arg
                245                 250                 255

Trp Arg Leu Val Gly Val Ala Pro Pro Arg Asp Trp Arg Ala Ala Leu
            260                 265                 270

Arg Glu Ala Met Arg Gln Leu Leu Pro Gly Gly Arg Leu Arg Asn Leu
        275                 280                 285

Thr Gly Thr
    290
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 350 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogK*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Met Ala Ser His Thr Ser Ala Thr Thr Asp Val Asn Ile Leu Val Thr
1               5                   10                  15

Gly Ala Val Gly Phe Ile Gly Ser Ala Tyr Val Arg Met Leu Leu Glu
            20                  25                  30

Asn Arg Ala Pro Gly Ala Gly Ala Pro Ala Val Arg Val Thr Val Leu
        35                  40                  45

Asp Lys Leu Thr Tyr Ala Gly Asn Leu Thr Asn Leu Asp Ala Val Arg
    50                  55                  60

Gly Asp Arg Leu Arg Phe Val Arg Gly Asp Ile Leu Asp Ala Glu Leu
65                  70                  75                  80

Val Asp Glu Leu Met Ala His Ser Asp Gln Val Val His Phe Ala Ala
                85                  90                  95

Glu Ser His Val Asp Arg Ser Ile Arg Ala Ala Asp Asp Phe Val Leu
            100                 105                 110

Thr Asn Val Val Gly Thr Gln Arg Leu Leu Asp Ala Ala Leu Arg His
        115                 120                 125

Gly Val Glu Pro Phe Val Leu Val Ser Thr Asp Glu Val Tyr Gly Ser
    130                 135                 140

Ile Ala Ser Gly Ser Trp Pro Glu Glu His Pro Leu Ser Pro Asn Ser
145                 150                 155                 160

Pro Tyr Ala Ala Ser Lys Ala Ser Ala Asp Leu Met Ala Phe Ala Cys
                165                 170                 175

His Arg Thr His Gly Leu Asp Val Arg Val Thr Arg Cys Ser Asn Asn
            180                 185                 190
```

Tyr Gly Pro Arg Gln His Pro Glu Lys Leu Ile Pro Arg Phe Val Thr
195 200 205

Asn Leu Leu Asp Gly Leu Pro Val Pro Leu Tyr Gly Asp Gly Arg Asn
210 215 220

Val Arg Glu Trp Leu His Val Glu Asp His Cys Arg Gly Val Asp Leu
225 230 235 240

Val Arg Thr Ala Gly Arg Pro Gly Gly Val Tyr His Ile Gly Gly Gly
245 250 255

Arg Glu Leu Ser Asn Arg Glu Leu Val Gly Met Leu Leu Glu Leu Cys
260 265 270

Gly Ala Asp Trp Ser Ser Val Arg His Val Pro Asp Arg Lys Gly His
275 280 285

Asp Leu Arg Tyr Ser Leu Asp Trp Gly Arg Ala Arg Glu Glu Leu Gly
290 295 300

Tyr Arg Pro Ala Arg Glu Phe Ser Ser Gly Leu Arg Ser Thr Val Gln
305 310 315 320

Trp Tyr Arg Glu Asn Arg Ser Trp Trp Glu Pro Leu Lys Arg Gly Val
325 330 335

Thr Ala Pro Gly Gly Thr Ser Thr Val Val Pro Gly Val Arg
340 345 350

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoaL*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Val Ser Ala Phe Asn Thr Gly Arg Thr Asp Asp Val Asp Glu Tyr
1 5 10 15

Ile His Pro Asp Tyr Leu Asn Pro Ala Thr Leu Glu His Gly Ile His
20 25 30

Thr Gly Pro Lys Ala Phe Ala Gln Leu Val Gly Trp Val Arg Ala Thr
35 40 45

Phe Ser Glu Glu Ala Arg Leu Glu Glu Val Arg Ile Glu Glu Arg Gly
50 55 60

Pro Trp Val Lys Ala Tyr Leu Val Leu Tyr Gly Arg His Val Gly Arg
65 70 75 80

Leu Val Gly Met Pro Pro Thr Asp Arg Arg Phe Ser Gly Glu Gln Val
85 90 95

His Leu Met Arg Ile Val Asp Gly Lys Ile Arg Asp His Arg Asp Trp
100 105 110

Pro Asp Phe Gln Gly Thr Leu Arg Gln Leu Gly Asp Pro Trp Pro Asp
115 120 125

Asp Glu Gly Trp Arg Pro
130

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 235 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoK*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Pro Asp Pro Gly Gly Pro Thr Thr Ala Glu Asn Leu Ser Lys Glu
1 5 10 15

Ala Val Arg Phe Tyr Arg Glu Gln Gly Tyr Val His Ile Pro Arg Val
20 25 30

Leu Ser Glu Thr Glu Val Thr Ala Phe Arg Ala Ala Cys Glu Glu Val
35 40 45

Leu Glu Lys Glu Gly Arg Glu Ile Ser Gly Ile Ala Leu Arg Leu Ala
50 55 60

Gly Ala Pro Leu Arg Val Tyr Ser Ser Asp Ile Leu Val Lys Glu Pro
65 70 75 80

Lys Arg Thr Leu Pro Thr Leu Val His Asp Asp Glu Thr Gly Leu Pro
85 90 95

Leu Asn Glu Leu Ser Ala Thr Leu Thr Ala Trp Ile Ala Leu Thr Asp
100 105 110

Val Pro Val Glu Arg Gly Cys Met Ser Tyr Val Pro Gly Ser His Leu
115 120 125

Arg Ala Arg Glu Asp Arg Gln Glu His Met Thr Ser Phe Ala Glu Phe
130 135 140

Arg Asp Leu Ala Asp Val Trp Pro Asp Tyr Pro Trp Gln Pro Arg Val
145 150 155 160

Ala Val Pro Val Arg Ala Gly Asp Val Val Phe His His Cys Arg Thr
165 170 175

Val His Met Ala Glu Ala Asn Thr Ser Asp Ser Val Arg Met Ala His
180 185 190

Gly Val Val Tyr Met Asp Ala Asp Ala Thr Tyr Arg Pro Gly Val Gln
195 200 205

Asp Gly His Leu Ser Arg Leu Ser Pro Gly Asp Pro Leu Glu Gly Glu
210 215 220

Leu Phe Pro Leu Val Thr Ala Gly Thr Arg Gln
225 230 235

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 390 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogD*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Met Arg Val Pro Gly Ser Cys Arg Thr Gly Gly Ile Met Arg Ala Leu
1               5                  10                  15

Phe Ile Thr Ser Pro Gly Leu Ser His Ile Leu Pro Thr Val Pro Leu
            20                  25                  30

Ala Gln Ala Leu Arg Ala Leu Gly His Glu Val Arg Tyr Ala Thr Gly
        35                  40                  45

Gly Asp Ile Arg Ala Val Ala Glu Ala Gly Leu Cys Ala Val Asp Val
    50                  55                  60

Ser Pro Gly Val Asn Tyr Ala Lys Leu Phe Val Pro Asp Asp Thr Asp
65                  70                  75                  80

Val Thr Asp Pro Met His Ser Glu Gly Leu Gly Glu Gly Phe Phe Ala
                85                  90                  95

Glu Met Phe Ala Arg Val Ser Ala Val Ala Val Asp Gly Ala Leu Arg
            100                 105                 110

Thr Ala Arg Ser Trp Arg Pro Asp Leu Val Val His Thr Pro Thr Gln
        115                 120                 125

Gly Ala Gly Pro Leu Thr Ala Ala Ala Leu Gln Leu Pro Cys Val Glu
    130                 135                 140

Leu Pro Leu Gly Pro Ala Asp Ser Glu Pro Gly Leu Gly Ala Leu Ile
145                 150                 155                 160

Arg Arg Ala Met Ser Lys Asp Tyr Glu Arg His Gly Val Thr Gly Glu
                165                 170                 175

Pro Thr Gly Ser Val Arg Leu Thr Thr Thr Pro Pro Ser Val Glu Ala
            180                 185                 190

Leu Leu Pro Glu Asp Arg Arg Ser Pro Gly Ala Trp Pro Met Arg Tyr
        195                 200                 205

Val Pro Tyr Asn Gly Gly Ala Val Leu Pro Asp Trp Leu Pro Pro Ala
    210                 215                 220

Ala Gly Arg Arg Arg Ile Ala Val Thr Leu Gly Ser Ile Asp Ala Leu
225                 230                 235                 240

Ser Gly Gly Ile Ala Lys Leu Ala Pro Leu Phe Ser Glu Val Ala Asp
                245                 250                 255

Val Asp Ala Glu Phe Val Leu Thr Leu Gly Gly Gly Asp Leu Ala Leu
            260                 265                 270

Leu Gly Glu Leu Pro Ala Asn Val Pro Val Val Glu Trp Ile Pro Leu
        275                 280                 285
```

```
Gly Ala Leu Leu Glu Thr Cys Asp Ala Ile Ile His His Gly Gly Ser
290                 295                 300

Gly Thr Leu Leu Thr Ala Leu Ala Ala Gly Val Pro Gln Cys Val Ile
305                 310                 315                 320

Pro His Gly Ser Tyr Gln Asp Thr Asn Arg Asp Val Leu Thr Gly Leu
                325                 330                 335

Gly Ile Gly Phe Asp Ala Glu Ala Gly Ser Leu Gly Ala Glu Gln Cys
            340                 345                 350

Arg Arg Leu Leu Asp Asp Ala Gly Leu Arg Glu Ala Ala Leu Arg Val
        355                 360                 365

Arg Gln Glu Met Ser Glu Met Pro Pro Pro Ala Glu Thr Ala Ala Lys
    370                 375                 380

Leu Val Ala Leu Ala Gly
385                 390
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 275 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoW*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Met Thr Val Leu Val Thr Gly Ala Thr Gly Asn Val Gly Arg His Val
1               5                   10                  15

Val Thr Gly Leu Leu Ala Ala Gly Arg Arg Val Arg Ala Leu Thr Arg
            20                  25                  30

Thr Pro Asp Arg Ser Gly Leu Pro Gly Gly Ala Glu Ile Thr Gly Gly
        35                  40                  45

Asp Leu Thr Arg Pro Glu Thr Tyr Glu Arg Met Leu Asp Gly Val Glu
    50                  55                  60

Ala Val Tyr Leu Phe Pro Val Pro Glu Thr Ala Ala Ala Phe Ala Gly
65                  70                  75                  80

Ala Ala Arg Arg Ala Gly Val Arg Arg Ile Val Val Leu Ser Ser Asp
                85                  90                  95

Ser Val Thr Asp Gly Thr Asp Thr Gly Gly His Arg Arg Val Glu Leu
            100                 105                 110

Ala Val Glu Asp Thr Gly Leu Glu Trp Thr His Val Arg Pro Gly Glu
        115                 120                 125

Phe Ala Leu Asn Lys Val Thr Leu Trp Ala Pro Ser Ile Arg Ala Glu
    130                 135                 140

Gly Val Val Arg Ser Ala Tyr Pro Asp Ala Arg Val Ala Pro Val His
145                 150                 155                 160

Glu Ala Asp Val Ala Ala Val Ala Val Thr Ala Leu Leu Lys Glu Gly
                165                 170                 175
```

```
His Ala Gly Arg Ala Tyr Ser Val Thr Gly Pro Gln Ala Leu Thr Gln
            180                 185                 190

Arg Glu Gln Val Arg Ala Val Gly Glu Gly Leu Gly Arg Ser Leu Ala
        195                 200                 205

Phe Val Glu Val Thr Pro Gly Gln Ala Arg Ala Asp Leu Thr Ala Gln
    210                 215                 220

Gly Leu Pro Ala Pro Ile Ala Asp Tyr Val Leu Ala Phe Gln Ala Gly
225                 230                 235                 240

Trp Thr Glu Arg Pro Ala Pro Ala Arg Pro Thr Val Arg Glu Val Thr
                245                 250                 255

Gly Arg Pro Ala Arg Thr Leu Ala Gln Trp Ala Ala Asp His Arg Ala
            260                 265                 270

Asp Phe Arg
        275
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: over 424 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snogE*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Val Arg Val Leu Leu Thr Ser Phe Ala Met Asp Ala His Phe Cys Thr
1               5                   10                  15

Ala Val Pro Leu Ala Trp Ala Leu Arg Ser Ala Gly His Glu Val Arg
            20                  25                  30

Val Ala Gly Gln Pro Ala Leu Thr Ser Thr Ile Thr Gly Ala Gly Leu
        35                  40                  45

Thr Ala Val Pro Val Gly Arg Asp His Thr His Gly Ser Leu Leu Gly
    50                  55                  60

Arg Val Gly Ser Asp Ile Leu Ala Leu His Asp Glu Ala Asp Tyr Leu
65                  70                  75                  80

Glu Ala Arg His Asp Ala Leu Gly Phe Glu Phe Leu Lys Gly His Asn
                85                  90                  95

Thr Val Met Ser Ala Leu Phe Tyr Ser Gln Ile Asn Asn Asp Ser Met
            100                 105                 110

Val Asp Asp Leu Val Asp Phe Ala Arg His Trp Arg Pro Asp Leu Val
        115                 120                 125

Val Trp Glu Pro Phe Thr Phe Ala Gly Ala Val Ala Ala Arg Ala Ser
    130                 135                 140

Gly Ala Ala His Ala Arg Leu Leu Ser Phe Pro Asp Leu Phe Leu Ser
145                 150                 155                 160

Thr Arg Arg Leu Phe Leu Glu Arg Met Ala Arg Gln Glu Pro Glu His
                165                 170                 175
```

```
His Asp Asp Thr Leu Ala Glu Trp Leu Asp Trp Thr Leu Gly Arg His
            180                 185                 190

Gly His Ser Phe Asp Glu Glu Ile Val Thr Gly Gln Trp Ser Ile Asp
        195                 200                 205

Gln Thr Pro Ala Pro Val Arg Leu Asp Ala Gly Gly Pro Thr Val Pro
    210                 215                 220

Met Arg Tyr Val Pro Tyr Ser Gly Leu Val Pro Thr Val Val Pro Asp
225                 230                 235                 240

Trp Leu Arg Arg Pro Pro Glu Arg Pro Arg Val Leu Val Thr Leu Gly
                245                 250                 255

Ile Thr Ser Arg Arg Val Lys Ser Phe Leu Ala Val Ser Val Asp Asp
            260                 265                 270

Leu Phe Glu Ala Val Ala Gly Leu Gly Val Glu Val Val Ala Thr Leu
        275                 280                 285

Asp Ala Asp Gln Arg Glu Leu Leu Gly Arg Val Pro Asp His Phe Arg
    290                 295                 300

Ile Val Glu His Val Pro Leu Asp Ala Val Leu Pro Thr Cys Ser Ala
305                 310                 315                 320

Ile Val His His Gly Gly Ala Gly Thr Trp Ser Thr Ala Ala Val Tyr
                325                 330                 335

Gly Val Pro Gln Val Ser Leu Gly Ser Met Trp Asp His Phe Tyr Arg
            340                 345                 350

Ala Arg Arg Leu Glu Glu Leu Gly Ala Gly Leu Arg Leu Pro Ser Gly
        355                 360                 365

Glu Leu Thr Ala Glu Gly Leu Arg Thr Arg Leu Glu Arg Val Leu Gly
    370                 375                 380

Glu Pro Ser Phe Gly Thr Ala Ala Gln Ala Leu Ser Asp Thr Ile Ala
385                 390                 395                 400

Ala Glu Pro Ser Pro Ser Glu Val Val Pro Val Leu Glu Glu Leu Thr
                405                 410                 415

Gly Arg His Arg Pro Gly Thr Arg
            420
```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *sno*L"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
Met Ser Thr Thr Ala Asn Lys Glu Arg Cys Leu Glu Met Val Ala Ala
1               5                   10                  15

Trp Asn Arg Trp Asp Val Ser Gly Val Val Ala His Trp Ala Pro Asp
            20                  25                  30
```

```
Val Val His Tyr Asp Asp Glu Asp Lys Pro Val Ser Ala Glu Glu Val
        35                  40                  45

Val Arg Arg Met Asn Ser Ala Val Glu Ala Phe Pro Asp Leu Arg Leu
    50                  55                  60

Asp Val Arg Ser Ile Val Gly Glu Gly Asp Arg Val Met Leu Arg Ile
65                  70                  75                  80

Thr Cys Ser Ala Thr His Gln Gly Val Phe Met Gly Ile Ala Pro Thr
                85                  90                  95

Gly Arg Lys Val Arg Trp Thr Tyr Leu Glu Glu Leu Arg Phe Ser Glu
            100                 105                 110

Ala Gly Lys Val Val Glu His Trp Asp Val Phe Asn Phe Ser Pro Leu
        115                 120                 125

Phe Arg Asp Leu Gly Val Val Pro Asp Gly Leu
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoO*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Met Ser Val Arg Thr Asp Gln Thr Ala Ala Pro Glu Asp Arg Ala Ala
1               5                   10                  15

Ala Thr Asp Pro Gly Phe Gly His Leu Tyr Ala Gln Val Gln Gln Phe
            20                  25                  30

Tyr Ala Arg Gln Met Gln Leu Leu Asp Ser Gly Ala Ala Glu Glu Trp
        35                  40                  45

Ala Ala Thr Phe Thr Glu Asp Gly Thr Phe Ala Arg Pro Ser Ser Pro
    50                  55                  60

Glu Pro Ala Arg Gly His Ala Glu Leu Ala Ala Gly Ala Arg Ala Ala
65                  70                  75                  80

Ala Glu Arg Leu Ala Ala Glu Gly Leu Ser His Arg His Val Ile Gly
                85                  90                  95

Met Thr Ala Val Arg Arg Glu Pro Asp Gly Ser Val Phe Val Arg Ser
            100                 105                 110

Tyr Ala Gln Val Phe Ala Thr Arg Arg Gly Glu Ala Pro Arg Leu His
        115                 120                 125

Leu Ile Cys Val Cys Glu Asp Val Leu Val Arg Glu Gly Pro Gly Leu
    130                 135                 140

Lys Val Arg Glu Arg Val Val Thr His Asp Ala
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoaF*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Val Arg Ala Met Thr Asp Ser Thr Gly Pro Arg Pro Val Pro Ala Met
1               5                  10                  15

Ser Pro Ala Pro Ser Pro Thr Pro Ser Pro Gly Pro Ala Pro Gly Ser
            20                  25                  30

Glu Pro Ala Pro Leu Ala Val Ile Val Thr Gly Gly Gly Ser Gly Ile
        35                  40                  45

Gly Arg Ala Thr Ala Arg Ala Phe Ala Ala Gln Gly Ala Lys Val Leu
    50                  55                  60

Val Val Gly Arg Thr Glu Asp Ala Leu Ala Gln Thr Ala Glu Gly Cys
65                  70                  75                  80

Ala Asp Met Arg Val Leu Val Ala Asp Val Ala Ser Pro Asp Gly Pro
                85                  90                  95

Gln Ala Val Val Asn Ala Ala Leu Arg Glu Phe Gly Arg Ile Asp Val
            100                 105                 110

Leu Val Asn Asn Ala Ala Val Ala Gly Met Glu Thr Leu Gln Thr Val
        115                 120                 125

Asp Arg Asp Ala Val Ala Arg Gln Phe Gly Thr Asn Leu Thr Ala Pro
    130                 135                 140

Leu Phe Leu Val Gln Ser Ala Leu Gly Ala Leu Glu Lys Ser Arg Gly
145                 150                 155                 160

Ile Val Val Asn Val Gly Thr Ala Ala Thr Leu Gly Leu Arg Ala Ala
                165                 170                 175

Pro Thr Gly Ala Leu Tyr Gly Ala Ser Lys Val Ala Leu Asp Tyr Leu
            180                 185                 190

Thr Arg Thr Trp Ala Val Glu Leu Ala Pro Arg Gly Ile Arg Val Val
        195                 200                 205

Gly Val Ala Pro Gly Val Ile Asp Thr Gly Ile Gly Val Arg Met Gly
    210                 215                 220

Met Thr Pro Glu Gly Tyr Arg Glu Phe Leu Thr Gly Met Gly Gly Arg
225                 230                 235                 240

Val Pro Val Gly Arg Val Gly Arg Pro Glu Asp Val Ala Trp Trp Ile
                245                 250                 255

Val Gln Leu Ala Arg Pro Glu Ala Gly Tyr Ala Thr Gly Met Val Val
            260                 265                 270

Pro Val Asp Gly Gly Leu Ser Leu Val
        275                 280
```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 190 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(D) OTHER INFORMATION: /note= "translate of *snoN*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
Val Gln Glu Thr Glu Pro Gly Val Pro Ala Asp Leu Pro Ala Glu Ser
1               5                  10                  15

Asp Pro Ala Ala Leu Glu Arg Leu Ala Ala Arg Tyr Arg Arg Asp Gly
            20                  25                  30

Tyr Val His Val Pro Gly Val Leu Asp Ala Gly Glu Val Ala Glu Tyr
        35                  40                  45

Leu Ala Glu Ala Arg Arg Leu Leu Ala His Glu Glu Ser Val Arg Trp
    50                  55                  60

Gly Ser Gly Ala Gly Thr Val Met Asp Tyr Val Ala Asp Ala Gln Leu
65                  70                  75                  80

Gly Ser Asp Thr Met Arg Arg Leu Ala Thr His Pro Arg Ile Ala Ala
                85                  90                  95

Leu Ala Glu Tyr Leu Ala Gly Ser Pro Leu Arg Leu Phe Lys Leu Glu
            100                 105                 110

Val Leu Leu Lys Glu Asn Lys Glu Lys Asp Ala Ser Val Pro Thr Ala
        115                 120                 125

Pro His His Asp Ala Phe Ala Phe Pro Phe Ser Thr Ala Gly Thr Ala
    130                 135                 140

Leu Thr Ala Trp Val Ala Leu Val Asp Val Pro Val Glu Arg Gly Cys
145                 150                 155                 160

Met Thr Phe Val Pro Gly Ser His Leu Leu Pro Asp Pro Asp Thr Gly
                165                 170                 175

Asp Glu Pro Trp Ala Gly Ala Phe Thr Arg Pro Gly Glu Ile
            180                 185                 190
```

Claims

1. Isolated and purified DNA fragment, which is the gene cluster for the anthracycline biosynthetic pathway of the bacterium *Streptomyces nogalater*, being included in a 10kb and a 7kb flanked *Bgl*II fragments of *S. nogalater* genome.

2. The DNA fragment according to claim 1, comprising the nucleotide sequence given in SEQ ID NO:1, or a sequence showing at least 80% homology to said sequence.

3. A recombinant DNA, which comprises the DNA fragment according to claim 1 or 2, cloned in a plasmid replicating in *Streptomyces*.

4. The recombinant DNA according to claim 3, which is the plasmid pSY15c, comprising a 1.4 kb *Bam*HI–*Sac*I fragment from the plasmid pSY42 and a 1.1 kb *Mlu*I–*Kpn*I fragment from the plasmid pSY43.

5. Plasmid pSY42, deposited in *S. lividans* strain TK24/pSY42 with the deposition number DSM 12451.

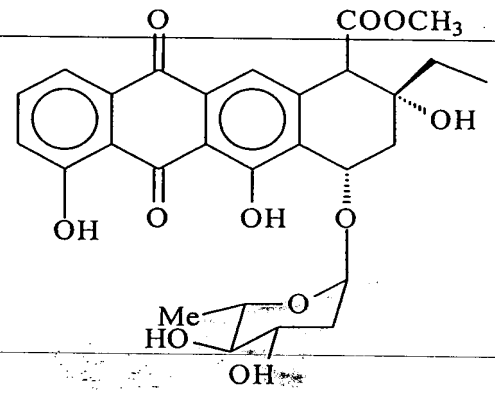
6. Plasmid pSY43, deposited in *S. lividans* strain TK24/pSY43 with the deposition number DSM 12452.

7. A process for the production of hybrid compounds, comprising transferring the DNA fragment according to claim 1 or 2 into a *Streptomyces* host, cultivating the recombinant strain obtained, and isolating the compounds produced.

8. The process according to claim 7, wherein the *Streptomyces* host is a *Streptomyces galilaeus* host.

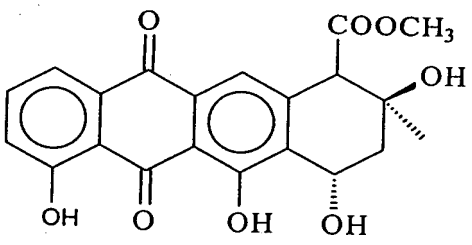
9. The process according to claim 8, wherein the *Streptomyces galilaeus* host is selected from the strains H026, H039, H063 and H075, which are mutant strains of *S. galilaeus* ATCC 31615.

10. The process according to claim 8, wherein an anthracycline is produced, which has the following formula I



(I)

11. The process according to claim 8, wherein an anthracyclinone is produced, which has the following formula II



(II)

12. A process for the production of hybrid compounds, comprising transferring at least one of the genes selected from the group consisting of *snog*J, *snog*A, *snoa*M, *snog*N, *snoa*G, *snog*C, *snog*K, *snoa*L, *sno*K, *snog*D, *sno*W, *snog*E, *sno*L, *sno*O and

*sno*aF into a *Streptomyces* host, said genes being derived from the DNA fragment of claim 1 or 2, cultivating the recombinant strain obtained, and isolating the compounds produced.

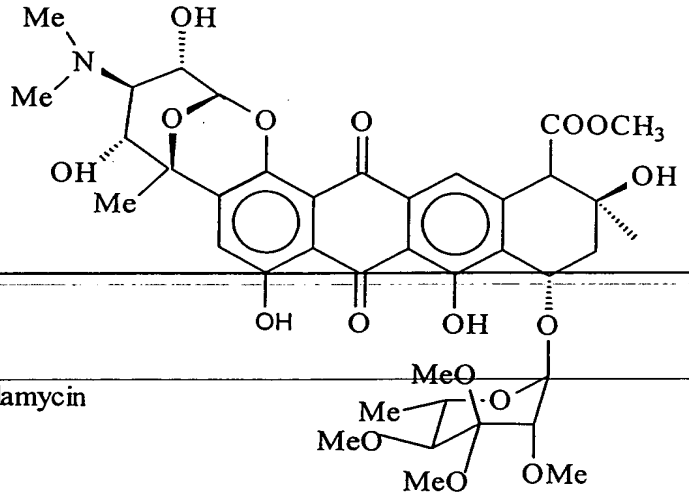
13. The process according to claim 12, wherein the gene *sno*aL encoding NAME cyclase is transferred into a *Streptomyces* host.

14. The process according to claim 12, wherein at least one of the genes *sno*gD and *sno*gE encoding glycosyl transferases is transferred into a *Streptomyces* host.

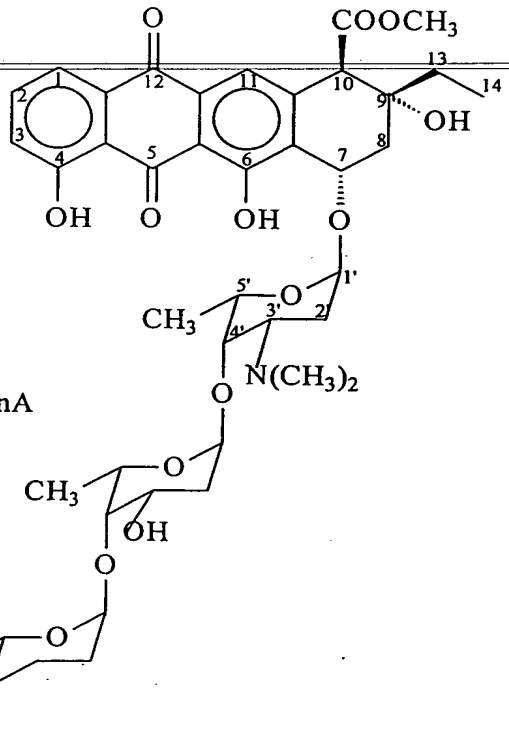
15. The process according to claim 12, wherein at least one of the genes *sno*gJ, *sno*gN, *sno*gC, *sno*gK and *sno*gA affecting the formation of nogalamine and nogalose is transferred into a *Streptomyces* host.

Abstract

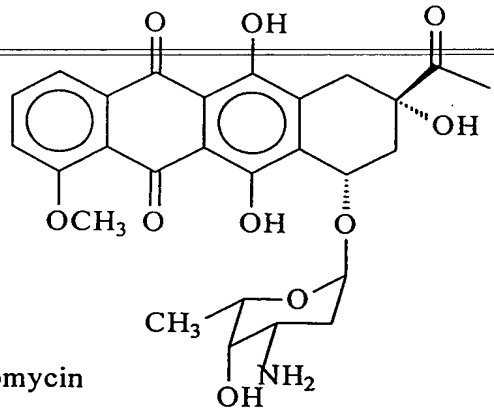
The present invention relates to the gene cluster for nogalamycin biosynthesis derived from *Streptomyces nogalater*, and the use of the genes therein to obtain novel hybrid antibiotics for drug screening.



Nogalamycin



AclacinomycinA



Daunomycin

Fig. 1

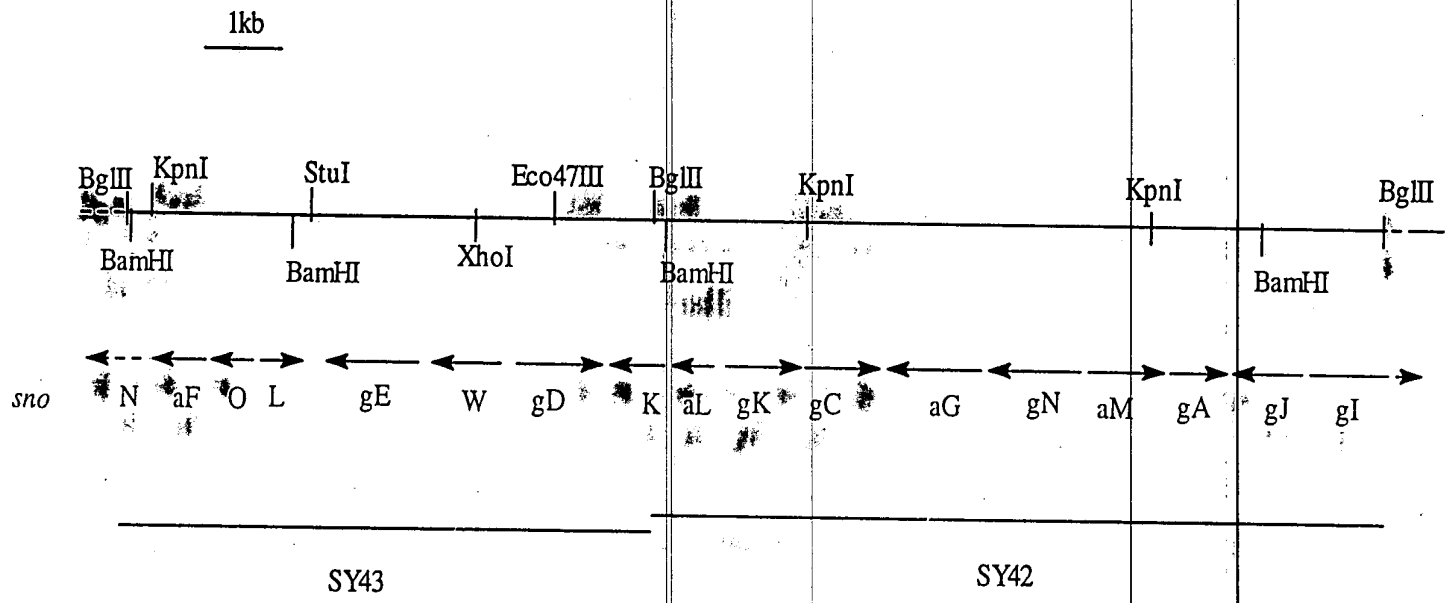

1kb
BglII
KpnI
StuI
Eco47III
BglII
KpnI
KpnI
BglII
BamHI
BamHI
XhoI
BamHI
BamHI
sno
N
aF
O
L
gE
W
gD
K
aL
gK
gC
aG
gN
aM
gA
gJ
gI
SY43
SY42

Fig. 2

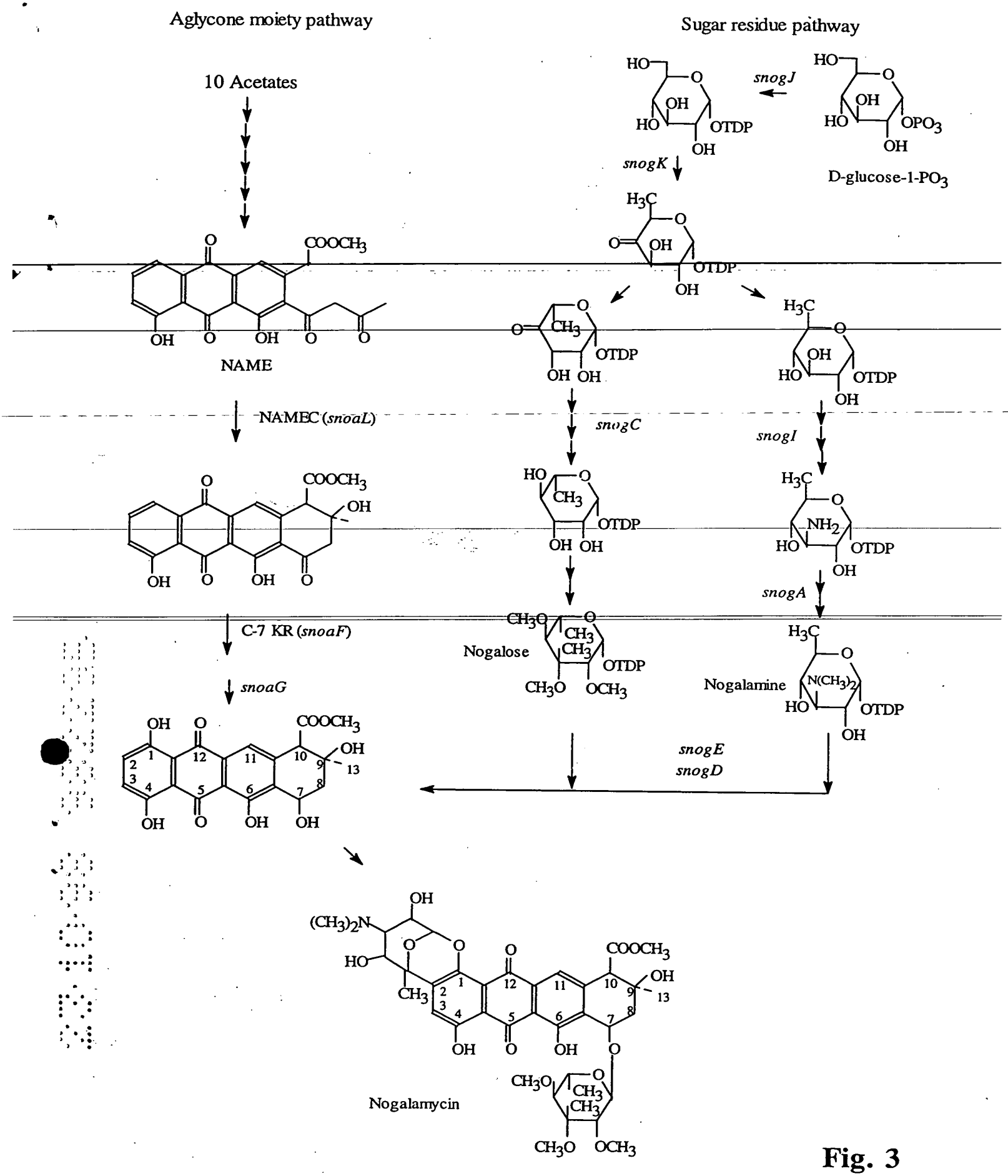

Aglycone moiety pathway
Sugar residue pathway
10 Acetates
snogJ
snogK
D-glucose-1-PO3
NAME
NAMEC (snoaL)
snogC
snogI
C-7 KR (snoaF)
snoaG
snogA
Nogalose
Nogalamine
snogE
snogD
Nogalamycin

Fig. 3

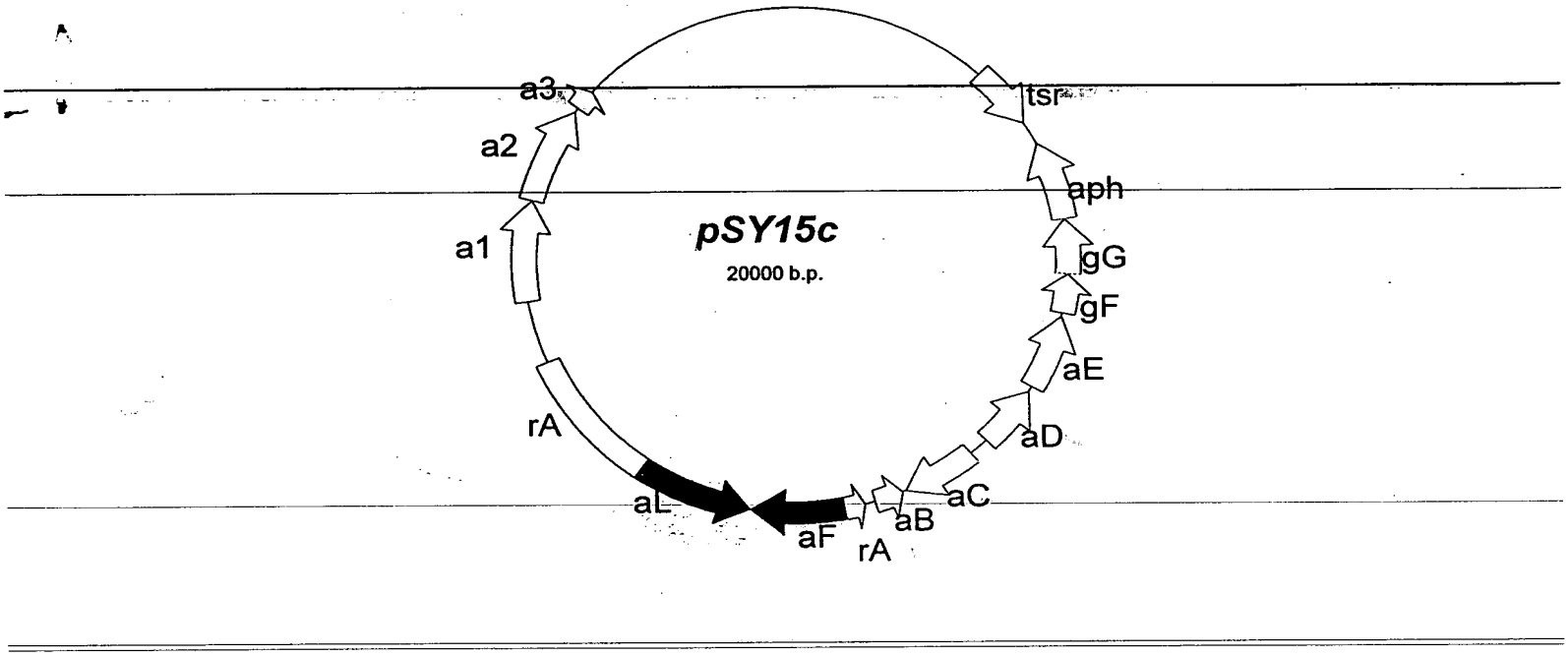


Fig. 4